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Movement and Persistence of [^{14}C]Imidacloprid in Sugar-Beet Plants Following Application to Pelleted Sugar-Beet Seed

Fahimeh Westwood,[1]* Kathy M. Bean,[1] Alan M. Dewar,[1] Richard H. Bromilow[2] & Keith Chamberlain[2]

[1] IACR-Broom's Barn, Higham, Bury St Edmunds, Suffolk IP28 6NP, UK
[2] IACR-Rothamsted, Harpenden, Herts. AL5 2JQ, UK

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Abstract: [^{14}C]Imidacloprid was applied to pelleted seeds of sugar beet which were then grown in pots of field soil. Leaves, roots and soil were analysed at intervals up to 97 days after planting and the distributions of parent compound and of several metabolites were quantified. At the first sampling, 21 days after application, parent imidacloprid was the main compound found in the leaves and its concentration averaged 15·2 μg g^{-1} fresh weight. By the 25-leaf stage, 97 days after sowing, the concentration of parent compound in the leaves had fallen to an average of 0·5 μg g^{-1}; the metabolites and parent compound in the leaves then represented respectively 44·5% and 4·5% of the total applied radioactivity. In the root at 97 days, parent imidacloprid and its metabolites together accounted for only 0·1% of the applied activity, whilst in the soil there was 23% of parent compound and 4% as metabolites.

The persistence of both parent imidacloprid and the olefinic metabolite, which has recently been shown to have higher aphicidal activity than the parent imidacloprid, explains the prolonged control of aphids observed with imidacloprid in both glasshouse and field trials. © 1998 SCI.

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Key words: imidacloprid; aphids; seed treatment; sugar beet

1 INTRODUCTION

Imidacloprid (Fig. 1, **1**) is a systemic nitroguanidine insecticide that was introduced to the sugar-beet crop in the UK in 1994.[1] It has a wide range of activity, giving good control of soil pests such as springtails, millipedes, symphylids and pygmy beetles[2–5] and foliar pests, such as aphids and leaf miners.[6–13] In the UK and elsewhere, imidacloprid is applied to the outside of pelleted sugar-beet seed as a film coating at the rate of 90 g AI per 100 000 seeds.[14] At this application rate, it is sufficiently persistent to control aphids for up to 10 weeks after sowing, with consequent reduction in virus yellows infection.[10] Lengthy persistence in soil has been found in other studies.[15]

The translocation of [^{14}C]imidacloprid in maize, cotton and winter wheat has been studied in detail.[16] Koester[17] studied the comparative metabolism of imidacloprid in suspension cultures of cells of several plant species, and found that the initial degradation pathway went mainly *via* hydroxylation of the imidazolidine ring, dehydration of which led to the olefinic form (Δ^4-imidazoline ring). It has recently been reported that this olefinic metabolite is indeed more insecticidal to *Myzus persicae* Sulz and *Aphis gossypi* Glov. than parent imidacloprid when fed *via* artificial sap in parafilm sachets.[18] However, little information is available on the metabolic pathway of imidacloprid in sugar-beet plants;

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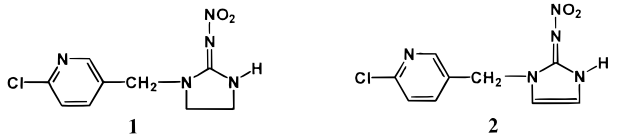

Fig. 1. Structures of imidacloprid (**1**) + olefinic metabolite (**2**).

such knowledge would aid understanding of the efficacy of aphid control.

This paper describes the movement and persistence of [^{14}C]imidacloprid applied to pelleted sugar-beet seed both within the plant and in the soil; a preliminary account of part of this work has been given previously.[19]

2 MATERIALS AND METHODS

2.1 Chemicals

2.1.1 [^{14}C]Imidacloprid

[*pyridinyl*-^{14}C-*methyl*]Imidacloprid with a radiochemical purity of 98% was acquired from Bayer plc and was used at a specific activity of 159 MBq mmole^{-1}.

2.1.2 Synthesis of olefinic metabolite (imidazoline analogue; Fig. 1, 2)

A solution of 2-nitroaminoimidazole (2·56 g, 0·02 mole) in dry *N*,*N*-dimethylformamide (20 ml) was cooled to 5°C and sodium hydride (60% dispersion in oil, 0·96 g, 0·024 mole) was added in portions with thorough stirring. After the addition was complete, the mixture was allowed to come to room temperature and stirred until no more hydrogen was evolved. The mixture was then cooled to 10°C and a solution of 2-chloro-5-chloromethylpyridine (3·25 g, 0·02 mole) in dry dimethylformamide (4 ml) was added dropwise. After 3 h further stirring at room temperature, the mixture was poured into iced water (150 ml). The crystalline brown solid which separated was collected and washed with water, followed by recrystallisation from ethanol with charcoal to give fine, off-white crystals (0·8 g) m.p. 189–191°C (dec), (lit. 186–189°C).[20] The [^{1}H] and [^{13}C]NMR spectra (400 MHz, $CDCl_3$, TMS) were consistent with the proposed structure for the olefinic metabolite.

2.2 Pot experiment and application of [^{14}C]imidacloprid to seed pellet

The soil (Ashley series: sand 52%, clay 24% and silt 24%) was collected from Little Lane field at IACR-Broom's Barn in Suffolk. The soil was partially air-dried (to a soil moisture of 10·6% w/w) and then passed through a 3-mm sieve. Pots of 21 cm diameter were filled with 4 kg of this soil and 3·2 g of ammonium nitrate (nutrient), was added. Prior to sowing, each pot received 100 ml of tap water.

Twenty-five sugar-beet seeds (*Beta vulgaris altissima* Doell., cv. Saxon), previously pelleted without insecticide by Germain's UK Ltd. and with a mean pelleted weight of 39·4($\pm$2·8) mg, were planted singly at 3 cm depth in each pot of soil. Before covering with soil, each pelleted seed was treated with 900 μg of [^{14}C]imidacloprid, applied as 150 μl of an aqueous dispersion of a 700 g kg^{-1} ws formulation, using a micropipette. The imidacloprid was allowed to be absorbed onto the pelleting material for approximately two hours before the seeds were covered, and the pots were first watered on the next day. The plants were subsequently grown in a glasshouse maintained at 17–21°C, and soil moisture was maintained at *c.* 40% (w/w). As the seed coating disintegrates once the seed germinates, it was thought that the behaviour of the externally applied [^{14}C]imidacloprid in these tests would be a fair representation of that arising from the commercial seed-coating procedure.

Five plants were harvested on each of four occasions 21, 49, 64 and 97 days after sowing when plants were at the 2-, 8-, 16- and 25-leaf stages respectively. The roots were cut off, thoroughly washed and weighed; the plant tops were cut into pairs of leaves (oldest pair labelled 1 + 2 etc) and stored separately. The plant parts were weighed and stored in a freezer at $-$20°C until analysis. The soil was thoroughly mixed and stored briefly at 5°C until analysis.

2.3 Measurement of [^{14}C]imidacloprid and metabolites in plant and soil samples

2.3.1 Leaves

Pairs of leaves were chopped and mixed, and duplicate samples (1·0 g) were extracted with acetonitrile + water (80 + 20 by volume; 20 ml) by homogenisation in an ultra-Turrax-T25 homogeniser for 10 min; the extract was then centrifuged at 10 000*g* for 15 min at 5°C to remove particulate matter. Duplicate aliquots of the supernatant solution (0·25 ml) were taken for measurement of total ^{14}C, scintillation fluid (Ultima Gold; 2 ml) being added and the radioactivity measured (10 min) using a Canberra-Packard TR1600 liquid scintillation counter (LSC) with quench correction by external standard. The remainder of the extract (19·75 ml) was evaporated to dryness in a rotary evaporator at a bath temperature of 50°C and the residue redissolved in dichloromethane (1·0 ml); duplicate aliquots (0·1 ml) were applied to 1 cm wide pre-absorbent strips on 19-channel thin-layer chromatography (TLC) plates coated with a 250-μm layer of silica-gel, fluorescent at 254 nm (Whatman). The plates were developed with dichloromethane + methanol (96 + 4, by volume). Plates were then scanned on a Berthold linear analyser

(10 to 60 min) with a 1-cm window and the total radioactivity obtained from the LSC was apportioned between the parent [^{14}C]imidacloprid and its metabolites. The efficiency of recovery of [^{14}C]imidacloprid added to control leaf samples at 45 μg g^{-1} was 96($\pm$2)%.

2.3.2 Roots

Roots were chopped and duplicate samples (1·0 g) were homogenised with acetonitrile + water (80 + 20 by volume; 10 ml), as for the leaves, and left overnight with stirring. After centrifugation, the solution was filtered into a 25-ml round-bottomed flask and evaporated to dryness on a rotary evaporator. The residue was triturated with acetonitrile (3 × 10 ml) to dissolve the imidacloprid compounds and leave the sucrose as a white precipitate. The combined acetonitrile extracts were evaporated to dryness, the residue dissolved in dichloromethane (1·0 ml) and an aliquot (0·1 ml) taken for TLC as above. The remaining solution was again evaporated, dissolved in acetonitrile (0·50 ml) and an aliquot (0·25 ml) taken for measurement of total ^{14}C by LSC (see Section 2.3.1). The efficiency of recovery of [^{14}C]imidacloprid added to roots at 90 μg g^{-1} was 67($\pm$3)% which was lower than other matrices due to the additional step of sucrose removal.

2.3.3 Soil

After being passed through a 3-mm sieve, duplicate soil samples (20 g) from each pot were extracted with acetonitrile + water (80 + 20 by volume; 20 ml) by orbital shaking overnight. After centrifugation and evaporation as above, the residue was redissolved in acetonitrile (2·0 ml). Duplicate aliquots (0·25 ml) were taken for LSC. The remaining solution was evaporated, redissolved in dichloromethane (1·0 ml) and an aliquot (0·1 ml) taken for TLC as above. The efficiency of recovery of [^{14}C]imidacloprid added to soil at 4·5 μg g^{-1} from soil was 99($\pm$0·5)%.

High-performance liquid chromatography (HPLC) was carried out directly on the centrifuged soil extracts, using samples (20 μl) injected onto a Hypersil 5 μm ODS (150 × 4·6 mm ID) column. The mobile phase was water + acetonitrile (75 + 25 by volume) adjusted to pH 3·5 with phosphoric acid. The flow rate was 1·0 ml min^{-1} and the eluate was monitored at 265 nm. The retention time of imidacloprid was 4·7 min, and the recovery efficiency from soil by this procedure was 98%.

2.4 Separation of metabolites by thin-layer chromatrography

Several metabolites were observed on the TLC plates of leaf extracts developed in dichloromethane + methanol (96 + 4 by volume), one of which corresponded in R_f value to the olefinic compound reported previously[17] and here available by synthesis. To confirm the presence of this metabolite, extracts of leaves sampled 64 days after sowing were co-chromatographed with the synthesised compound in two additional solvent systems, ethyl acetate + toluene + methanol + acetic acid (80 + 20 + 20 + 1 by volume) and ethyl acetate + 2-propanol + water (65 + 23 + 12 by volume), and scanned as previously described.

3 RESULTS AND DISCUSSION

3.1 Plant growth

The emergence after 19 days of plants from the sugar-beet seeds treated with [^{14}C]imidacloprid was 84%, and from the control seeds was similar at 88%. The mean fresh weights for the tops and roots grown from the imidacloprid-treated seeds are given in Table 1. The plants were very small at the first sampling, and had insufficient roots for analysis.

3.2 Distribution and metabolism of [^{14}C]imidacloprid in the sugar-beet plants

3.2.1 Parent imidacloprid in leaves

Analyses from the duplicate samples were on average within $\pm$ 8·5%. The highest concentration of parent imidacloprid was at the two-leaf stage (21 days), reaching 15·2 μg g^{-1} (Fig. 2) with a total of 18·7 μg plant^{-1} (Fig. 3). At the second harvest, 49 days after sowing, a total of 44·3 μg of parent imidacloprid was detected, mostly in the oldest six leaves; concentrations in these leaves were up to 3 μg g^{-1}, although only 0·5 μg g^{-1} was detected in the youngest heart leaves. After 64 days, a total of 47·2 μg plant^{-1} was found; the concentration of parent compound was less than 1·2 μg g^{-1} in all leaves, and there was little difference between them. After 97 days the first two leaves had died and fallen off the plant, but a total of 40·7 μg plant^{-1} of parent compound was still detected. Concentrations in individual leaves were less than 0·5 μg g^{-1}, with the least found in the oldest and youngest leaves. Nonetheless, all the sampled plants contained parent imidacloprid in their leaves even at 97 days after planting, consistent with the

TABLE 1
Weights of Tops and Roots of Individual Sugar-Beet Plants

Time after sowing (days)	*Weight of plant part (g) ($\pm$SD)*	
	Tops	*Roots*
21	1·22 ($\pm$0·3)	<0·1
49	40 ($\pm$15)	7 ($\pm$4)
64	86 ($\pm$6)	15 ($\pm$5)
97	141 ($\pm$19)	75 ($\pm$12)

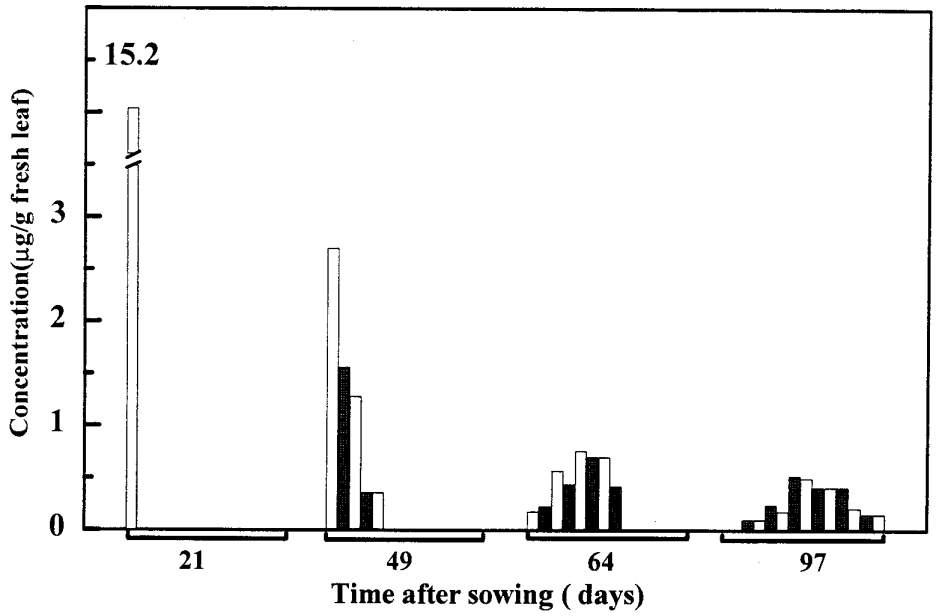

Fig. 2. Concentration of parent imidacloprid in pairs of leaves; oldest pair 1 to youngest pair (up to 13), left to right on each sampling date. Columns are unshaded for the odd-numbered leaf pairs and hatched for the even-numbered; note that at day 97 the oldest leaf pair had senesced.

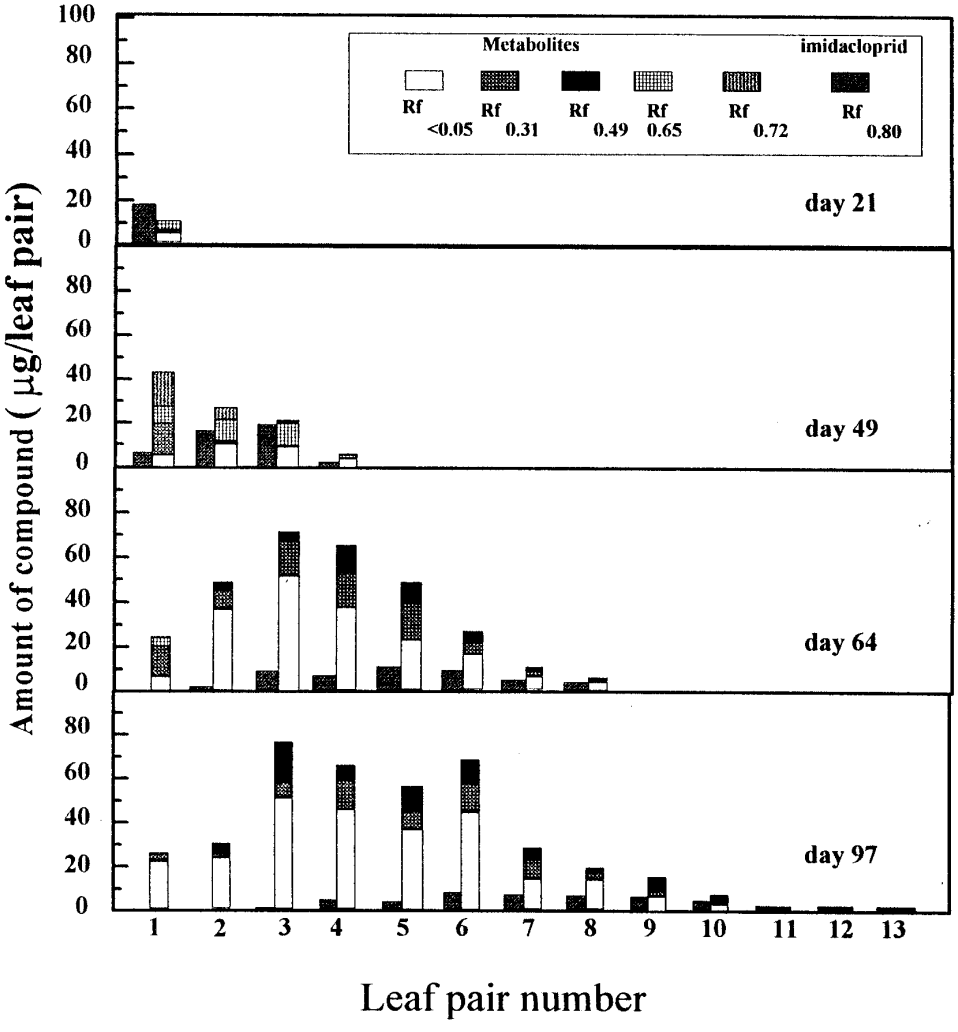

Fig. 3. Distribution and amounts of parent imidacloprid and major metabolites (olefinic metabolite has $R_f = 0{\cdot}49$) in sugar-beet leaves at different times after sowing.

long persistence in soil and hence continual availability for uptake by the roots.

Whilst the behaviour of parent imidacloprid was the prime objective of this study, scanning of the TLC plates also revealed the presence of several metabolites (Fig. 3, amounts of metabolites given as imidacloprid equivalents). Indeed, even by 49 days after sowing, the metabolites made up the major portion of the radioactivity extracted from the leaves. By day 97, the metabolites in the leaves represented 44·5% of the applied radioactivity compared to 4·5% for the parent imidacloprid. Thus imidacloprid was quite rapidly metabolised in the sugar beet plants.

3.2.2 Measurement and identification of metabolites from [^{14}C]imidacloprid in leaves

Aliquots of the extracts subjected to additional TLC in several solvent systems showed major metabolites with the R_f values given in Table 2.

Imidacloprid itself ran faster than the olefinic metabolite and was well separated from other metabolites. Only three minor metabolites were found in the 21- and 49-day tops, with very little of the olefinic metabolite observed. However, by 64 and 97 days after application, the main metabolites were the very polar fraction, thought to include 6-hydroxynicotinic acid, together with smaller but still appreciable amounts of the olefinic metabolite and a slightly slower-running unidentified metabolite that may be the monohydroxylated-imidazolidine compound.[17] Other metabolites were only observed at low levels and were not identified; 6-chloronicotinic acid had been reported as a metabolite in a cell suspension[17] but was not observed here (R_f = 0·14 in dichloromethane + methanol (96 + 4 by volume)).

The olefinic compound, being insecticidally active, was the main metabolite of interest in this study. Its averaged concentrations in the leaves are compared to those of imidacloprid in Table 3. It can be seen that, whilst only parent imidacloprid is likely to be controlling aphids at 21 and 49 days, by 64 and 97 days the olefinic metabolite was probably an important factor in giving prolonged aphid control as the concentrations of parent imidacloprid had by then declined. This might be especially so as the amounts of olefinic metabolite were greater in the older leaves than those of imidacloprid itself (Fig. 3), and so would help to control late-colonising aphids on the outer leaves

TABLE 2
Thin-Layer Chromatography on Silica Gel of Imidacloprid and its Major Metabolites in Sugar-Beet Leaves

Solvent[a]	R_f *values of imidacloprid*, its olefinic† and other metabolites*[b]					
1	<0·05	0·31	0·49†	0·65	0·73	0·80*
2	0·17	0·23	0·50†	n.d.	0·75	0·80
3	0·17	0·39	0·57†	n.d.	0·76	0·67*

[a] 1. Dichloromethane + methanol (96 + 4, by volume).
2. Ethyl acetate + toluene + methanol + acetic acid (80 + 20 + 20 + 1, by volume).
3. Ethyl acetate + 2-propanol + water (65 + 23 + 12, by volume).
[b] n.d. Not detected.

TABLE 3
Imidacloprid and its Olefinic Metabolite in Sugar Beet Leaves

	Mean concentration (μg g^{-1} fresh wt)			
	Time after planting (days)			
Compound	*21*	*49*	*64*	*97*
Imidacloprid	15·2	1·2	0·55	0·5
Olefinic metabolite	0·0	0·13	0·43	0·3

3.2.3 Imidacloprid and metabolites in roots

The amounts of parent imidacloprid found in the roots were 1·3, 1·7 and 0·08 μg at 49, 64 and 97 days after sowing respectively (the roots at 21 days were too small to be analysed). Thus the amounts of imidacloprid found in roots were always very small, less than 0·2% of that applied to the seed coating; likewise, only trace amounts of metabolites were observed in roots. The very small amount of imidacloprid found here at 97 days is in agreement with Rouchaud *et al.*[15] who found no imidacloprid in sugar-beet roots at harvest 210 days after sowing.

3.3 Persistence of imidacloprid in the soil

Most of the extracted ^{14}C co-chromatographed on TLC with parent imidacloprid, and only small amounts (<5% of that extracted from soil) of metabolites were observed, in agreement with previous work.[21,22] The amounts of imidacloprid recovered were 60, 48, 38 and 23% of that applied at sampling times 21, 49, 64 and 97 days after sowing respectively (Fig. 4): agreement between duplicate samples was generally ±10%. These results were confirmed by the HPLC measurements. Thus imidacloprid was present in soil and available for uptake by the sugar-beet plants throughout the 97 days of the experiment.

3.4 Summary of the distribution of [^{14}C]imidacloprid

The distribution of parent imidacloprid and its extractable metabolites between soil and above-ground parts of the sugar-beet plants is summarised in Fig. 4 (amounts in roots were negligible and have thus been excluded). It can be seen that much of the imidacloprid

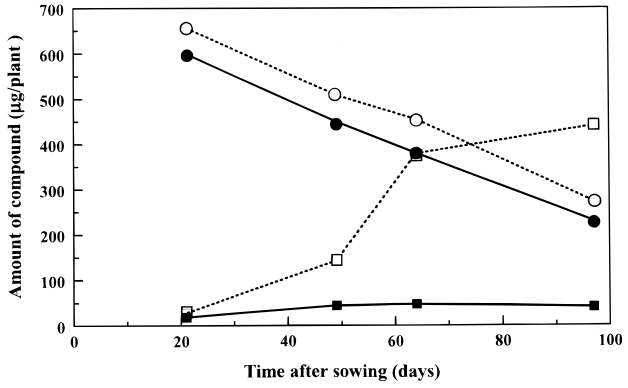

Fig. 4. Distribution of imidacloprid (900 μg applied per seed pellet) and its extractable metabolites between (■, □) leaves and (●, ○) soil; closed symbols represent parent imidcloprid and open symbols imidacloprid plus metabolites.

remained in the soil, with not more than 5·3% of that applied being found as parent imidacloprid in the plant leaves on any of the four sampling dates; nonetheless around half or slightly more of the applied imidacloprid was eventually taken up by the plants from the seed pellet and accumulated in the leaves as metabolites. Thus uptake is efficient and this can be explained by the physicochemical properties of imidacloprid. It is quite a polar compound, with log K_{ow} 0·57 (K_{ow} is the 1-octanol/water partition coefficient), and so its sorption to soil is rather weak; it is thus largely available in the soil water for uptake by plant roots, for which process the lipophilicity is also favourable.[23] Furthermore, such polarity leads to low retention by tissues such as roots and hence to rapid transport in the xylem to the margins and interveinal spaces of the leaves[23,24] as observed by Stein-Donecke.[16]

4 CONCLUSIONS

Much of the imidacloprid derived from the pelleting material around the sugar-beet seeds found its way to the soil, where it was moderately persistent with 23% of that applied being found in soil 97 days after sowing. The maximum amount of parent imidacloprid found in the plant tops was 5·3% of that applied at 64 days, and this declined slightly to 4·5% at 97 days. Concentrations of imidacloprid in the leaves were highest early in the experiment with up to 15·2 μg g^{-1} at 21 days, declining to around 0·5 μg g^{-1} at 97 days. The prolonged availability in soil for uptake is consistent with the sustained control of the aphid *M. persicae* in the field. Amounts found in roots were always very small. Only traces of metabolites were found in soil and in roots, whilst metabolites comprised the major portion of extractable ^{14}C from the leaves at and beyond 49 days; one was identified as the olefinic compound (Δ^4-imidazoline ring), with tentative assignments of the monohydroxylated imidacloprid and 6-hydroxynicotinic acid. The presence of appreciable concentrations of the olefinic metabolite in the 64- and 97-day samples is of particular interest as this compound has been shown to be very much more active against aphids than imidacloprid.[18] These results thus help explain the effectiveness of imidacloprid applied as a seed treatment in controlling the aphid *Myzus persicae* over a prolonged period sufficient to significantly reduce infection with virus yellows.[25]

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